IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: TONG, et al.

U.S. Serial No. 09/938,391 Group Art Unit: 1636

Filed: August 24, 2001 Examiner: Sumesh Kaushal, Ph.D.

For: METHODS AND COMPOSITIONS FOR DIAGNOSING AND TREATING DISORDERS INVOLVING ANGIOGENESIS

Attorney Docket No: 3153.00234

DECLARATION

I, Kendall King, do hereby say that:

1. I am an expert in the field of animal and human health, specifically with regard to molecular biology, having 18 years of experience in the field.

2. I have reviewed in detail the presently pending patent application and claims. Further, I have reviewed in detail the outstanding Office Action issued September 26, 2003, Paper Number 16.

3. I have reviewed the rejections of claims 1-11 under 35 U.S.C. § 101. According to the Office Action, claims 1-11 lack a specific asserted utility or a well-established utility as required under 35 U.S.C. § 101. More specifically, the Office Action holds that the specification fails to disclose that the claimed nucleic acid sequences encode a polypeptide that inhibits angiogenesis in a canine and there is a lack of evidence that establishes that the claimed nucleic acid sequences encode a polypeptide that has any anti-angiogenic activity *in vivo*. Moreover, the Office Action holds that there is only 60.7% sequence similarity between the claimed nucleotide sequence and a sequence related to human XVIII collagen (AN:AF018082) and that there are limited, if any, areas of conservation between the two sequences. In conclusion, the Examiner holds that one skilled in the art would not readily attribute any particular canine endostatin-like activity encoded by the claimed nucleic acid sequence or variants thereof in view of the low sequence similarity and the lack of sequence conservation therein.

4. I have reviewed the rejections of claims 1-11 under 35 U.S.C. § 112. More specifically, the Office Action holds that the specification fails to disclose that the claimed nucleic acid sequences encode a polypeptide that inhibit angiogenesis in a canine.

5. The Office Action holds that the specification does teach that the addition of the proposed canine-endostatin polypeptide inhibits the stimulating effect of bFGF on CPAE cells *in vitro*.

6. Based on the disclosure of the present application together with information known to those of skill in the art, the claimed nucleic acid sequence is both useful and enabled under 35 U.S.C. § 101 and 35 U.S.C. § 112, respectively. More specifically, the claimed nucleic acid sequence encodes for an endostatin that (1) inhibits endothelial cell proliferation and (2) has demonstrable *in vitro* and *in vivo* anti-angiogenic activity.

7. By way of background, the present invention relates to polynucleotide sequences that encode polypeptides that are associated with the regulation of angiogenesis. Angiogenesis is defined as the growth or sprouting of new blood vessels from existing vessels. Under normal physiological conditions in adults, angiogenesis takes place only in very restricted situations such as hair growth and wound healing. Unregulated angiogenesis has gradually been recognized to be responsible for a wide range of disorders such as cancer. Angiogenesis is required by solid tumors for their growth and metastasis. A tumor usually begins as a single aberrant cell that can proliferate only to a size of a few cubic millimeters due to the distance from available capillary beds and it can stay "dormant" without further growth and dissemination for a long period of time. Some tumor cells then switch to an angiogenic phenotype to activate endothelial cells, which proliferate and mature into new capillary blood vessels. These newly formed blood vessels are not only for continued growth of the primary tumor, but also for the dissemination and re-colonization of metastatic tumor cells.

8. As is well known in the art, one of the most potent angiogenesis inhibitors is endostatin. Endostatin is a proteolytic fragment of the

larger protein known as collagen XVIII. Endostatin has also been shown to specifically inhibit endothelial cell proliferation (i.e., inhibit new blood vessels from forming and thus inhibit angiogenesis).

9. The present invention is directed towards nucleotide sequences and polypeptides that have been isolated from canine genes. The claimed nucleotide sequences are derived from RNA from canine liver tissue cells. Primers based on consensus sequences from human, mouse, and chicken cells were utilized to amplify a region of the canine collagen XVIII cDNA.

10. The isolated canine endostatin was then successfully transfected into human cells. (See specification, page 70, line 31 to page 72, line 5). Moreover, detection of canine endostatin occurred by immunofluorescence and immunoanalysis. (See specification, page 71 and Figures 7 and 8). The results set forth in the present application prove that the expression of canine endostatin occurs. To further study the effects of the canine endostatin, inhibition of endothelial cell proliferation was also studied.

11. The instant specification, which the Office Action confirms, clearly demonstrates that the disclosed amino acid sequence, when recombinantly expressed, is able to inhibit the proliferation of calf pulmonary artery endothelial (C-PAE) cells. It is well documented in the prior art that recombinantly-expressed human and murine endostatin also inhibit the proliferation and migration of C-PAE cells (Dhanabal, et al., (1999a); Dhanabal, et al., *Cancer Res* 59: 189-197 (1999b)). Those studies also establish that this anti-proliferative activity is anti-angiogenic activity (O'Reilly, 1999; Dhanabal, 1999b).

12. As shown in the example section of the specification, inhibition of endothelial cell proliferation did occur (i.e., a demonstrable anti-angiogenic effect). Immunoflorescence and immunoblot assays confirm that the protein localized to the secretory pathway and was secreted into media. Canine endostatin was also shown to specifically inhibit endothelial proliferation at a level comparable to its murine counterpart. Since some tumor cells can switch to angiogenic phenotype to activate endothelial cells, which proliferate and mature

into new capillary blood vessels, inhibiting the growth of endothelial cells would help reduce new blood vessel growth. By preventing or ameliorating at least one symptom of the disorder (i.e., growth and dissemination of tumor cells through endothelial cells resulting in new capillary blood vessels), the presently claimed invention has utility with regard to an anti-angiogenic effect.

13. As is well-known in the art, and as set forth and proven in Figure 6 of the present application, a large degree of homology exists between the canine, human, mouse, and chicken endostatins. As set forth above, it is well established in the art that endostatin is a C-terminal fragment of type XVIII collagen (O'Reilly, et al., *Ce//,* 88: 277-285 (1997) and Dhanabal, et al., *Biochem Biophys Res Comm,* 258: 345~352 (1999)). With respect to homology, it is of greater relevance and importance that amino acid sequence comparisons should be made as opposed to nucleotide sequence comparisons. More specifically, amino acid identity (i.e., exact matches between sequences) was analyzed using the BestFit alignment program (Univ. of Wisconsin Genetics Computer Group). The claimed amino acid sequence is in fact 85.1, 83.7, and 76.1% *identical* to human, murine, and chicken endostatins, respectively. By comparison, human and murine endostatin are 85.6% identical to each other. Also, all cysteine residues are conserved between the disclosed amino acid sequence and the other three, which predicts that the overall tertiary structure is conserved (Dhanabal, et al.). Additionally, ClustalX alignment (Thompson, et al., *Nuc* Ac *Res,* 24: 4876-4882 (1997)) of the four sequences confirms that the disclosed amino acid sequence is most closely related to the human and murine sequences.

14. Thus, when taking into account the high degree of identity between the claimed amino acid sequence and other endostatin protein sequences, the anti-proliferative activity of the present invention, and the relationship between anti-proliferative and anti-angiogenic activities of the human and murine endostatin proteins, it is established that an anti-angiogenic function

can be ascribed to the disclosed amino acid sequence. These facts support the asserted utility and enablement of the claimed invention.

15. In view of the above and based on facts well known in the art and research, the claimed nucleotides and polypeptides are useful and enabled by the present specification. Moreover, due to the above facts and knowledge of those of skill in the art, the claimed nucleotide sequences encode for an endostatin that has *in vitro* and *in vivo* anti-angiogenic activity.

The undersigned declares further that all statements made here and of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereof.

_____
Kendall King

Dated: February ___, 2003

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15. In view of the above and based on facts well known in the art and research, the claimed nucleotides and polypeptides are useful and enabled by the present specification. Moreover, due to the above facts and knowledge of those of skill in the art, the claimed nucleotide sequences encode for an endostatin that has *in vitro* and *in vivo* anti-angiogenic activity.

The undersigned declares further that all statements made here and of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereof.

Kendall King

Dated: February 25, 2003

```
 BESTFIT of: bf.5876.1.fsn.gcg  check: 1416  from: 1  to: 183

bf.5876.1

 to: bf.5876.2.fsn.gcg  check: 552  from: 1  to: 184

bf.5876.2

 Symbol comparison table: /gcgsys/gcg9/gcgcore/data/rundata/blosum62.cmp
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     Length Weight:      4   Average Mismatch: -2.003

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             Ratio:  4.613               Gaps:      0
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                .         .         .         .         .
      1 HSHRDFQPVLHLVALNSPLSGGMRGIRGADFQCFQQARAVGLAGTFRAFL 50  hu Endostatin
        |.|.||| ||||||||||  ||||||||||||||||||| ||||||||||
      1 HTHQDFQLVLHLVALNSPQPGGMRGIRGADFQCFQQARAAGLAGTFRAFL 50  ca Endostatin
                .         .         .         .         .
     51 SSRLQDLYSIVRRADRAAVPIVNLKDELLFPSWEALFSGSEGPLKPGARI 100
        ||||||||||||||||  ||:|||:||.|||||||||||||| |||||||
     51 SSRLQDLYSIVRRADRTGVPVVNLRDEVLFPSWEALFSGSEGQLKPGARI 100
                .         .         .         .         .
    101 FSFDGKDVLRHPTWPQKSVWHGSDPNGRRLTESYCETWRTEAPSATGQAS 150
        |||||:|||.|| ||.|||||||||.|||||:||||||||||.||||||
    101 FSFDGRDVLQHPAWPRKSVWHGSDPSGRRLTDSYCETWRTEAPAATGQAS 150
                .         .         .
    151 SLLGGRLLGQSAASCHHAYIVLCIENSFMTA 181
        ||| |||| | |||| ||::||||||| ||.
    151 SLLAGRLLEQEAASCRHAFVVLCIENSVMTS 181
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bf.8050.1

 to: bf.8050.2.fsn.gcg  check: 552  from: 1  to: 184

bf.8050.2

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     Length Weight:      4   Average Mismatch: -2.003

           Quality:    831             Length:    184
             Ratio:  4.516               Gaps:      0
 Percent Similarity: 87.500   Percent Identity: 83.696

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                  : =   2
                  . =   1

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                .         .         .         .         .
      1 HTHQDFQPVLHLVALNTPLSGGMRGIRGADFQCFQQARAVGLSGTFRAFL 50   mu Endostatin
        ||||||| ||||||||.|  ||||||||||||||||||| ||.|||||||
      1 HTHQDFQLVLHLVALNSPQPGGMRGIRGADFQCFQQARAAGLAGTFRAFL 50   ca Endostatin
                .         .         .         .         .
     51 SSRLQDLYSIVRRADRGSVPIVNLKDEVLSPSWDSLFSGSQGQLQPGARI 100
        |||||||||||||||  ||:|||:|||| |||:.|||||:|||.|||||
     51 SSRLQDLYSIVRRADRTGVPVVNLRDEVLFPSWEALFSGSEGQLKPGARI 100
                .         .         .         .         .
    101 FSFDGRDVLRHPAWPQKSVWHGSDPSGRRLMESYCETWRTETTGATGQAS 150
        |||||||||.|||||.|||||||||||||| :|||||||||   ||||||
    101 FSFDGRDVLQHPAWPRKSVWHGSDPSGRRLTDSYCETWRTEAPAATGQAS 150
                .         .         .
    151 SLLSGRLLEQKAASCHNSYIVLCIENSFMTSFSK 184
        |||.||||||.|||| ..::|||||||| ||||||
    151 SLLAGRLLEQEAASCRHAFVVLCIENSVMTSFSK 184
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bf.8199.1

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bf.8199.2

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     Length Weight:      4   Average Mismatch: -2.003

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             Ratio:  4.076               Gaps:      0
 Percent Similarity: 79.891   Percent Identity: 76.087

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                  . =   1

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                .         .         .         .         .
      1 HVHQDFQPALHLVALNTPLSGGMRGIRGADFQCFQQARQVGLAGTFRAFL 50   chick Endostatin
        | |||||  |||||||.|  ||||||||||||||||||  ||||||||||
      1 HTHQDFQLVLHLVALNSPQPGGMRGIRGADFQCFQQARAAGLAGTFRAFL 50   ca Endostatin
                .         .         .         .         .
     51 SSRLQDLYSIVRRADRTAVPIVNLRDEVLFSNWEALFTGSEAPLRAGARI 100
        ||||||||||||||||| ||:|||||||||| .|||||.|||  |: ||||
     51 SSRLQDLYSIVRRADRTGVPVVNLRDEVLFPSWEALFSGSEGQLKPGARI 100
                .         .         .         .         .
    101 LSFDGRDILQDSAWPQKSIWHGSDAKGRRLPESYCEAWRTDERGTSGQAS 150
         ||||||:||  |||.||:|||||  ||||| :|||| |||:    .||||
    101 FSFDGRDVLQHPAWPRKSVWHGSDPSGRRLTDSYCETWRTEAPAATGQAS 150
                .         .         .
    151 SLSSGKLLEQSASSCQHAFVVLCIENSFMTAAKK 184
        || .|:|||| |.||.||||||||||| ||.  |
    151 SLLAGRLLEQEAASCRHAFVVLCIENSVMTSFSK 184
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bf.9063.1

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bf.9063.2

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     Length Weight:      4   Average Mismatch: -2.003

           Quality:    840             Length:    181
             Ratio:  4.641               Gaps:      0
 Percent Similarity: 87.293   Percent Identity: 85.635

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                   : =   2
                   . =   1

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                .         .         .         .         .
       1 HSHRDFQPVLHLVALNSPLSGGMRGIRGADFQCFQQARAVGLAGTFRAFL 50   huEndostatin
         |.|.|||||||||||||.|||||||||||||||||||||||||.|||||||
       1 HTHQDFQPVLHLVALNTPLSGGMRGIRGADFQCFQQARAVGLSGTFRAFL 50   muEndostatin
                .         .         .         .         .
      51 SSRLQDLYSIVRRADRAAVPIVNLKDELLFPSWEALFSGSEGPLKPGARI 100
         |||||||||||||||| .|||||||||.| |||:.|||||:| |.|||||
      51 SSRLQDLYSIVRRADRGSVPIVNLKDEVLSPSWDSLFSGSQGQLQPGARI 100
                .         .         .         .         .
     101 FSFDGKDVLRHPTWPQKSVWHGSDPNGRRLTESYCETWRTEAPSATGQAS 150
         |||||:|||||| ||||||||||||.|||| ||||||||||   ||||||
     101 FSFDGRDVLRHPAWPQKSVWHGSDPSGRRLMESYCETWRTETTGATGQAS 150
                .         .         .
     151 SLLGGRLLGQSAASCHHAYIVLCIENSFMTA 181
         ||| |||| | |||||..||||||||||||.
     151 SLLSGRLLEQKAASCHNSYIVLCIENSFMTS 181
```

CLUSTAL W (1.82) multiple sequence alignment

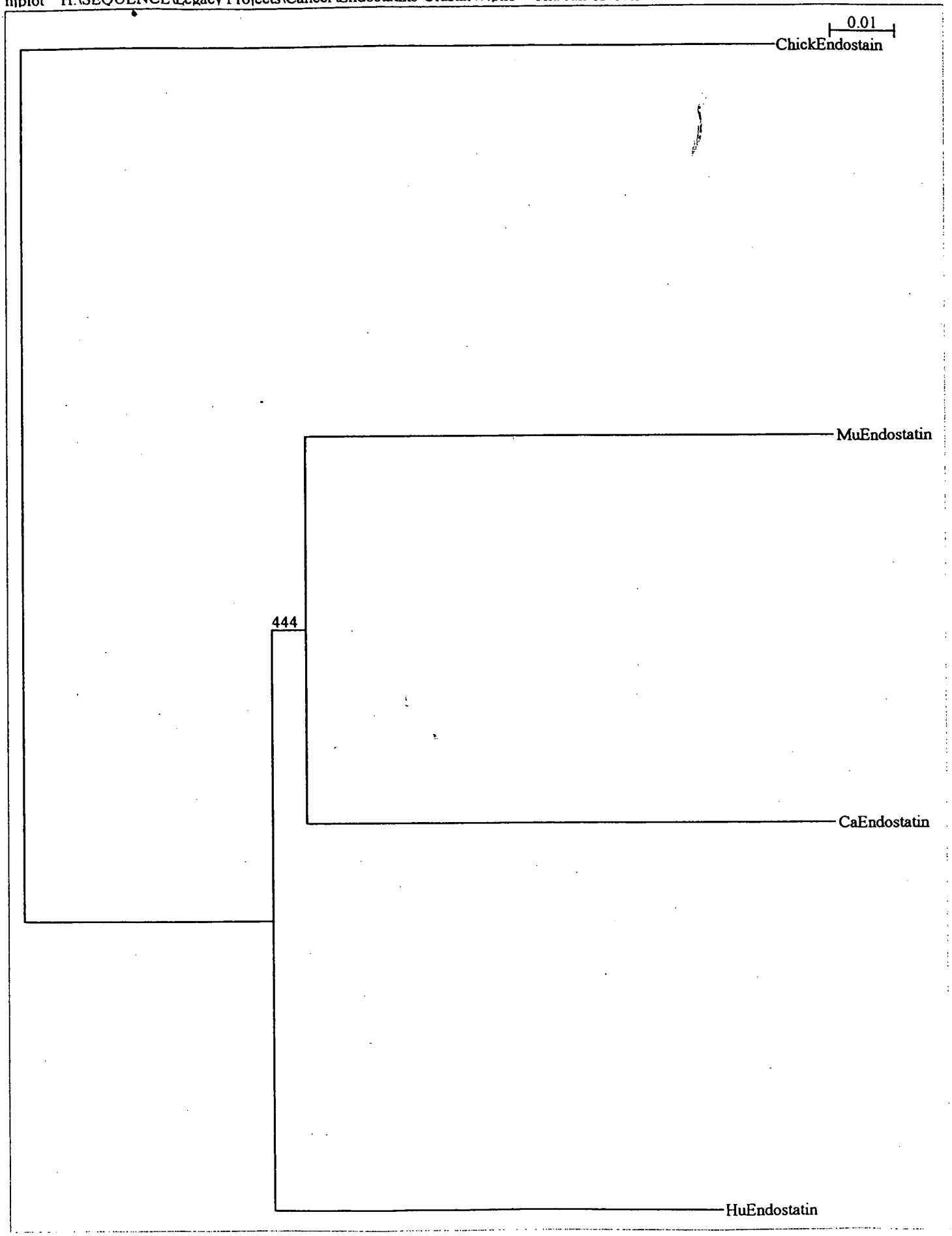
```
CaEndostatin        HTHQDFQLVLHLVALNSPQPGGMRGIRGADFQCFQQARAAGLAGTFRAFLSSRLQDLYSI 60
MuEndostatin        HTHQDFQPVLHLVALNTPLSGGMRGIRGADFQCFQQARAVGLSGTFRAFLSSRLQDLYSI 60
HuEndostatin        HSHRDFQPVLHLVALNSPLSGGMRGIRGADFQCFQQARAVGLAGTFRAFLSSRLQDLYSI 60
ChickEndostain      HVHQDFQPALHLVALNTPLSGGMRGIRGADFQCFQQARQVGLAGTFRAFLSSRLQDLYSI 60
                    * *:*** .*******:* .************ ****** .**:*****************

CaEndostatin        VRRADRTGVPVVNLRDEVLFPSWEALFSGSEGQLKPGARIFSFDGRDVLQHPAWPRKSVW 120
MuEndostatin        VRRADRGSVPIVNLKDEVLSPSWDSLFSGSQGQLQPGARIFSFDGRDVLRHPAWPQKSVW 120
HuEndostatin        VRRADRAAVPIVNLKDELLFPSWEALFSGSEGPLKPGARIFSFDGKDVLRHPTWPQKSVW 120
ChickEndostain      VRRADRTAVPIVNLRDEVLFSNWEALFTGSEAPLRAGARILSFDGRDILQDSAWPQKSIW 120
                    ****** .**:***:**:* ..*::**:**:. *:.****:****:*:*:..:**:**:*

CaEndostatin        HGSDPSGRRLTDSYCETWRTEAPAATGQASSLLAGRLLEQEAASCRHAFVVLCIENSVMT 180
MuEndostatin        HGSDPSGRRLMESYCETWRTETTGATGQASSLLSGRLLEQKAASCHNSYIVLCIENSFMT 180
HuEndostatin        HGSDPNGRRLTESYCETWRTEAPSATGQASSLLGGRLLGQSAASCHHAYIVLCIENSFMT 180
ChickEndostain      HGSDAKGRRLPESYCEAWRTDERGTSGQASSLSSGKLLEQSASSCQHAFVVLCIENSFMT 180
                    ****..**** :****:***:  .::****** .*:** *.*:**:::::******.**

CaEndostatin        SFSK 184
MuEndostatin        SFSK 184
HuEndostatin        ASK- 183
ChickEndostain      AAKK 184
                    : .
```

= conserved Cys residues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0.01
ChickEndostain
MuEndostatin
444
CaEndostatin
HuEndostatin